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Multi-drug resistant *Bacteroides fragilis* recovered from blood and severe leg wounds caused by an improvised explosive device (IED) in Afghanistan

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ABSTRACT

This report summarizes the case of a 23 year-old otherwise healthy male that was injured in an improvised explosive device (IED) blast in support of Operation Enduring Freedom (OEF). He sustained bilateral open tibia and fibula fractures in the setting of being exposed to water contaminated with raw sewage. Despite long-term carbapenem therapy, the patient's wounds were repeatedly noted to have purulent drainage during surgical debridement and cultures from these wounds were persistently positive for *Bacteroides fragilis*. Apparent clinical failure persisted despite the addition of metronidazole to his regimen and an eventual trial of tigecycline.

Susceptibility testing of the *B. fragilis* isolate was performed and resistance to penicillin, clindamycin,metronidazole, cefoxitin, meropenem, imipenem, piperacillin/tazobactam, and tigecycline was confirmed. The presence of a *nimE* gene on a potentially transferrable plasmid was also confirmed by plasmid sequencing. The only antibiotics that displayed *in vitro* susceptibility were moxifloxacin and linezolid. These antibiotics were initiated in combination with aggressive irrigation and serial surgical debridement. Conversion to left-sided internal fixation became feasible and his left lower extremity was salvaged without residual evidence of infection. The patient completed an eight week course of combination moxifloxacin and linezolid therapy without adverse event.

This *B. fragilis* isolate displayed simultaneous high-level resistance to multiple antibiotics routinely utilized in anaerobic infections. This was evidenced by clinical failure, *in vitro* susceptibility testing, and demonstration of genes associated with resistance mechanisms. This case warrants review not only due to the rarity of this event but also the potential implications regarding anaerobic infections in traumatic wounds and the success of a novel treatment regimen utilizing combination therapy with moxifloxacin and linezolid.

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1. Introduction

Bacteroides species are normally commensal in human colonic microbiota; however, infections with these bacteria can be associated with significant morbidity and mortality. The most commonly isolated Gram-negative anaerobic pathogen, *Bacteroides fragilis*, has potent virulence factors and has been associated with a variety of clinical syndromes including intra-abdominal sepsis and necrotizing skin and soft tissue infections. *B. fragilis* also has an underappreciated ability to develop several different mechanisms of resistance to universally utilized antimicrobials due to mobile genetic elements [1]. Susceptibility profiles can vary widely between different geographical locations and medical institutions. In the United States, multi-drug resistance is rare and anaerobic susceptibility testing is usually only pursued for specific surveillance purposes or due to extraordinary clinical circumstances.

In 2004, a national survey of the susceptibility patterns of *B. fragilis* was conducted in the United States. 5225 clinical isolates from 10 different medical centers were reviewed. Resistance to carbapenems and beta-lactam plus beta-lactamase inhibitor

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combinations was found in less than 1% of the total isolates while only one isolate in this report was resistant to metronidazole. However, rates of resistance to carbapenems and beta-lactam plus beta-lactamase inhibitor combinations have been increasing in more recent reviews [2]. In 2005, Wareham et al. [3] reported the isolation of a *B. fragilis* strain (WI1) that was simultaneously resistant to carbapenems, beta-lactam plus beta-lactamase inhibitor combinations, and metronidazole in a patient who ultimately died from complications related to anaerobic sepsis. Subsequent genetic analysis of this isolate confirmed the presence of a carbapenemase gene (*cfiA*), macrolide-lincosamide-streptograminin resistance associated gene *ermF*, a plasmid-located tetracycline resistance *tetQ*, and increased efflux pump gene expression among several mechanisms of resistance [4]. 12 (6.9%) of 175 clinical *B. fragilis* isolates in another review from the United Kingdom were positive for carbapenemase gene (*cfiA*) indicating that these isolates could potentially convert to high-level resistance when placed under selective pressure [5]. In blood stream *B. fragilis* isolates from National Taiwan University Hospital rates of resistance to ampicillin plus sulbactam, clindamycin, and carbapenems have also been found to be increasing over time [6]. Given the clinical importance of appropriate empiric antimicrobial selection for the treatment of anaerobic infections, these reports of resistance in *B. fragilis* isolates are particularly worrisome.

2. Case report

The patient in this review was a 23 year-old previously healthy United States Army soldier who sustained multiple injuries in an improvised explosive device (IED) blast in Afghanistan during the summer of 2009. He was riding in an armored patrol when the explosion occurred. The vehicle he was in rolled over into a local stream believed to be contaminated by raw sewage. There were known open sewer systems in the area and the patient himself reported visible pollution. He was submerged in water up to his neck for several minutes while awaiting initial medical care. His injuries included a C5/C6 cervical fracture, a right femoral fracture, and bilateral open tibia and fibula fractures. By the time he arrived at Landstuhl Regional Medical Center (LRMC) in Germany two days after this initial injury his lower extremity wounds were noted to have foul odor. He underwent multiple surgeries while at LRMC to include right femoral intramedullary nail placement, external fixation of his bilateral tibia and fibula fractures, a left lower extremity fasciotomy, and several wound irrigation and debridement procedures. Meropenem 1 g intravenously every 8 h was initiated empirically shortly after his admission to LRMC and antibiotic beads containing vancomycin and tobramycin were placed intra-operatively. Wound cultures grew both an extended spectrum beta-lactamase (ESBL) *Escherichia coli* and *B. fragilis*.

After his arrival at Walter Reed Army Medical Center (WRAMC) in the United States approximately one week after his initial injury, the patient's bilateral lower extremity wounds continued to produce purulent drainage despite serial irrigation and debridement and continuation of broad-spectrum antibiotic therapy with meropenem. *B. fragilis* bacteremia was transiently detected on admission to WRAMC although *B. fragilis* was not found on several subsequent blood cultures. Metronidazole 500 mg intravenously every 6 h was added to his antibiotic regimen, but despite this intervention the patient's right lower extremity could not be salvaged and he required amputation below the right knee three weeks into his WRAMC hospital course. In order to salvage his left leg, internal fixation of his left tibia fracture was completed and continuous wound irrigation with Dakin's solution was initiated. The predominant organism from intra-operative wound cultures remained *B. fragilis*. Due to refractory anaerobic wound infection despite nearly four weeks of meropenem therapy, the patient was transitioned to empiric tigecycline 1 gm intravenously every 12 h and anaerobic susceptibility testing was pursued.

2.1. Microbiology

The patient's initial wound cultures from his left lower extremity were positive for an ESBL *E. coli* and *B. fragilis*. He had an isolated blood culture one week later that was also positive for *B. fragilis*, although several follow up blood cultures were negative indicating that this bacteremia was transient. In addition, antibiotic susceptibility testing of his blood stream isolate revealed a phenotypically different strain that was susceptible to carbapenems. However, a total of eight wound cultures from serial irrigation and debridement procedures from bilateral lower extremities were positive for *B. fragilis*. This occurred while the patient was on meropenem and metronidazole therapy.

The susceptibility testing performed by Quest Diagnostics by "E" test and at the R.M. Alden Research Laboratory by the agar dilution method [7] on one of the patient's *B. fragilis* wound isolates from WRAMC is summarized in Table 1.

A retrospective analysis of his very first wound *B. fragilis* isolate by the R.M Alden Research Laboratory revealed identical high-level multi-drug resistance (MDR). The isolate was also analyzed for plasmid content and found to contain two plasmids: a 5.5 kb plasmid identical to pHAG isolated from a multi-drug resistant *B. fragilis* in London UK [4] and the Class III plasmid pBFB35 [8] and also an 8.3 kb plasmid (designated pWAL610) with confirmed similarity to the sequenced fragment of pBF388c, an 8.3 kb plasmid present in a metronidazole resistant *B. fragilis* isolated in Kuwait [9]. The 8.3 kb pWAL610 was fully sequenced and annotated (Fig. 1). The nitroimidazole resistance gene *nimE* is 100% identical to the *nimE* gene in pBF388c. A transposase, 99% identical to tranposase Bf6 of pBF388c, was identified and also located within an IS4 family insertion sequence.

2.2. Clinical course

Based on ongoing clinical evidence of infection and available susceptibility data tigecycline was discontinued after only a few days of therapy in favor of combination therapy with both moxifloxacin and linezolid. Moxifloxacin 400 mg intravenously every day and linezolid 600 mg intravenously twice daily were initiated and transitioned to an oral route of administration when his scheduled surgeries became less frequent. Both agents were utilized simultaneously due to concerns regarding demonstration

Table 1
Minimum inhibitory concentrations (MIC) in μg/ml of 12 antibiotic/antibiotic combinations for a Bacteroides fragilis wound isolate.

Antibiotic	MIC	Interpretation
Penicillin	> 256	Resistant
Ampicillin and sulbactam	> 128/64	Resistant
Piperacillin and tazobactam	> 64/4	Resistant
Clindamycin	> 128	Resistant
Metronidazole	> 64	Resistant
Cefoxitin	> 64	Resistant
Imipenem	> 32	Resistant
Meropenem	> 32	Resistant
Tigecycline	> 16	Resistant
Chloramphenicol	> 32	Resistant
Moxifloxacin	< 0.5	Susceptible
Linezolid	< 2	Susceptible

Susceptibility testing was performed by E-test (which antibiotics) and by agar dilution (which antibiotics).

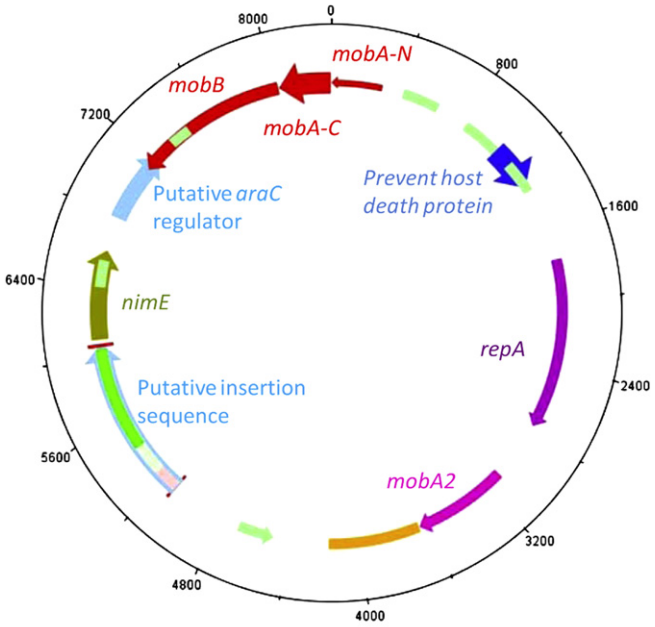

Fig. 1. Artemis DNA plotter image of the 8.3 kb pWAL610 isolated and sequenced from the patient's *B. fragilis* isolate (WAL610). Circular tracks from outside to inside: 1, DNA co-ordinates; 2, forward strand putative coding sequences; 3, reverse strand putative coding sequences; 4, insertion sequence IS*Bf6*; 5, inverted repeats.

of high-level resistance as well as the simple lack of additional therapeutic options. Due to the depth of invasion and the presence of hardware, this antibiotic regimen was continued for an 8 week total duration without adverse effect and with complete resolution of signs and symptoms of infection. The patient's left lower extremity was saved and upon discharge from the hospital he was ambulating with the use of right lower extremity prosthesis.

3. Discussion

B. fragilis infections are generally endogenous, arising from the patient's own normal microbiota [10]. In this case, however, it seems likely that the infection was environmentally acquired which is highly unusual. Although *B. fragilis* is an obligatory anaerobic bacterium that cannot be cultivated in the presence of oxygen, it is aero-tolerant and able to survive for a several hours under aerobic conditions [11]. This case demonstrates how underlying problems regarding the lack of infrastructure in Afghanistan have impacted the medical support mission in Operation Enduring Freedom (OEF). In the urban areas of Afghanistan specifically it is estimated that only 12–23% of the population has access to a safe source of drinking water. With the notable exception of one wastewater treatment facility near the city of Kabul, wastewater collection and treatment are virtually non-existent throughout the country. As a result, open sewer systems are commonplace [12]. The confirmation of identical high-level resistance in the patient's first clinical isolate of *B. fragilis* suggests an environmental source of the MDR isolate as opposed to treatment-induced resistance. It is highly likely that the MDR *B. fragilis* arose from within the local human population and that the water the patient was exposed to was contaminated through this population's utilization of an open sewer system. The presence of a 5.5 plasmid identical in sequence to a plasmid that is widespread across Europe and partial identity to an 8.3 plasmid identified in isolates from Europe and Kuwait is also intriguing.

B. fragilis and taxonomically related *Bacteroides* species are usually resistant to benzylpenicillin, other penicillins and, with the exception of cephamycins such as cefoxitin, many cephalosporins. Classically, resistance to penicillin in *B. fragilis* is associated with the production of active site serine beta-lactamases distantly related to Ambler's Class A. These beta-lactamases are susceptible to the inhibitory effects of compounds such as clavulanic acid, sulbactam and tazobactam. Resistance genes, for example *cepA*, may be plasmid or chromosomally located, and on mobile or mobilisable genetic elements. Similarly, the *cfxA* gene which encodes a cephalosporinase that degrades cephalosporins, penicillins and cefoxitin is located on a mobilisable transposon, *Tn4555*. Some *B. fragilis* strains produce a zinc requiring metallo-beta-lactamase of Ambler's Class B, associated with resistance to cephamycins and carbapenems. These enzymes are resistant to the beta-lactam inhibitors and are associated with the chromosomally encoded gene *cfiA* (*ccrA*). Furthermore, macrolide (e.g. erythromycin), lincosamide (e.g. clindamycin) and streptogramin (e.g. pristinamycin, virginiamycin) resistance is transferrable within and between *Bacteroides* spp. via conjugative plasmid transfer and also chromosomally located self-transmissible conjugative elements [13].A wide range of metronidazole resistance mechanisms have been described (e.g. Patel et al., 2009; Steffens et al., 2010) in addition to the *nim* (A-F) encoding genes [14–16]. Unlike the MDR *B. fragilis* WI1 isolated in London in 2004, the isolate presented in this review possesses a *nim* gene (*nimE)* located on a potentially mobile plasmid. The WI1 isolate demonstrated resistance to metronidazole without expression of a *nim* gene, an ability attributed to this isolate's enhanced efflux pump mechanisms. Similarly, efflux mechanisms and modifications of topoisomerase genes can contribute to fluoroquinolone resistance [4]. Of course, the WAL610 isolate did not demonstrate resistance to fluoroquinolones *in vitro* and a combination of both moxifloxacin and linezolid was the final successful treatment regimen.

Linezolid is traditionally thought of as a therapeutic option for resistant Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*. However, it may also have efficacy in treatment of resistant anaerobic infections. In one review evaluating the minimal inhibition concentrations (MIC's) for linezolid among 265 different anaerobes, the MIC range for the *B. fragilis* group was found to consistently range between 2 and 4 mg/l. The authors discovered no linezolid-resistant strains, suggesting that linezolid may be a potential candidate for the treatment of anaerobic infections [17]. Although most Gram-negative bacteria are resistant to linezolid as a result of endogenous efflux activity, another review examining MIC's for linezolid among *Bacteroides* species reported MIC's between 4 and 8 mg/L [18]. Of note, the WI1 case patient was also treated with linezolid therapy with apparent microbiological cure although the patient did not survive [3].

The reports over the past several years regarding drug-resistant *B. fragilis* isolates in clinical settings is concerning, especially when an isolate such as WAL610 reveals the ability to simultaneously express multiple different mechanisms of resistance. WAL610 closely resembles the MDR WI1 in this regard, although the mechanism of metronidazole resistance appears to be different. Further genetic analysis of the MDR *B. fragilis* WAL610 is underway to determine the mechanisms that generate resistance to the other antibiotics. While MDR *B. fragilis* have not previously been reported in United States, the very existence of isolates such as WAL610 and WI1 raises serious concerns regarding the eventual spread of resistance mechanisms and selection of empiric antibiotic therapy in anaerobic infections. The Clinical Laboratory Standards Institute (CLSI) does not recommend routine susceptibility testing for clinically encountered anaerobes although testing is recommended to

"assist in management of infection in individual patients with serious or life-threatening infections" and "persistence of infection despite adequate treatment with an appropriate therapeutic regimen" [7]. The diagnosis of a multi-drug resistant isolate in this case was delayed in part due to the time it took to arrange adequate anaerobic susceptibility testing through appropriate reference laboratories.

4. Conclusion

MDR *B. fragilis* is a truly rare clinical pathogen in the United States, although *Bacteroides* species certainly exhibit chromosomally mediated and plasmid-encoded resistance mechanisms and readily acquire resistance genes via mobile and mobilisable genetic elements. There are reports of increasing rates of resistance to beta-lactam and beta-lactamase inhibitor combinations and carbapenems throughout the world. While there has been much attention placed on MDR pathogens associated with traumatic war wounds from Iraq and Afghanistan such as *Acinetobacter baumannii* and the ESBL producing enterobactereciae, MDR *B. fragilis* should also be considered in patients with consistent exposure history especially when empiric antibiotics are failing. Furthermore, the oxazolidinone antibiotic linezolid represents a novel and potentially useful therapeutic option for resistant anaerobic infections.

Disclosures

There are no conflicts of interest. The views expressed in this case report are those of the authors and do not necessarily reflect the official policy or position of the Department of the Army, Department of Defense, or the U.S. Government.

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